10054725 BIOSIS NO.: 199598509643
RNase H is responsible for the non-specific inhibition of in vitro translation by 2'-O-alkyl chimeric **oligonucleotides**: High affinity or selectivity, a dilemma to design antisense oligomers.
AUTHOR: Larrouy Beatric; Boiziau Claudine; Sproat Brian; Toulme Jean-Jacques(a)
AUTHOR ADDRESS: (a)INSERM U 386, Lab. Biophysique Moleculaire, Univ. Bordeaux II, 33076 Bordeaux Cedex**France
JOURNAL: Nucleic Acids Research 23 (17):p3434-3440 1995
ISSN: 0305-1048
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Ribonuclease H (RNase H) which recognizes and cleaves the RNA strand of mismatched RNA-DNA heteroduplexes can induce non-specific effects of antisense **oligonucleotides**. In a previous paper (Larrouy et al. (1992), Gene, 121, 189-194), we demonstrated that ODN1, a phosphodiester 15mer targeted to the AUG initiation region of alpha-globin mRNA, inhibited mRNA. Specificity was restored by using MP-ODN2, a methylphosphonate-phosphodiester sandwich analogue of ODN1, which limited RNase H **activity** on non-perfect hybrids. We report here that 2'-O-alkyl RNA-phosphodiester DNA sandwich analogues of ODN1, with the same phosphodiester window as MP-ODN2, are non-specific inhibitors of globin synthesis in wheat germ extract, whatever the substituent (methyl, allyl or butyl) on the 2'-OH. These sandwich oligomers induced the cleavage of non-target beta-globin RNA sites, similarly to the unmodified parent oligomer ODN1. This is likely due to the increased affinity of 2'-O-alkyl-ODN2 chimeric oligomers for both fully and partly complementary RNA, compared to MP-ODN2. In contrast, the fully **modified** 2'-O-methyl analogue of ODN1 was a very effective and highly specific antisense sequence. This was ascribed to its inability (i) to induce RNA cleavage by RNase H and (II) to physically prevent the elongation of the polypeptide chain.

7/3,AB/6 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

09831905 BIOSIS NO.: 199598286823
Interaction of RNase H from E. coli with **modified** **oligonucleotide** hybrid duplexes. I. Duplexes containing modification in furanose moiety.
AUTHOR: Krynetskaya N F; Alekseev Ya I; Belkov V M; Ibrahim H C H; Tashlitskii V N; Oretskaya T S; Shabarova Z A
AUTHOR ADDRESS: Fac. Chem., M.V. Lomonosov Mosc. State Univ., Leninskie gory, Moscow 119899**Russia
JOURNAL: Bioorganicheskaya Khimiya 20 (11):p1218-1225 1994
ISSN: 0132-3423
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: Russian; Non-English
SUMMARY LANGUAGE: Russian; English

ABSTRACT: The influence of oligodeoxyribonucleotide probes containing 1-(D-beta-2'-deoxy-threo-pentofuranosyl)thymine or 1-(D-beta-2'-deoxy-2'-fluoro-pentofuranosyl)uracil on the ability of the hybrid duplexes to interact with RNase H from E. coli was studied. A kinetic approach was used to measure of the modification effect. The hybrid duplex, prA-18/d(TTflU)-6TT, was shown not to interact with RNase H, whereas prA-18/d(xTTT)-6 inhibited the RNase H **activity** (K-i=0.67 mkM). The thermostability of the **modified** duplexes was

estimated. The present technique may lead to the use of some **modified oligonucleotides** as antisenses.

7/3,AB/7 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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08899953 BIOSIS NO.: 199396051454
Evaluation of 2'-**modified oligonucleotides** containing 2'-deoxy gaps as antisense inhibitors of gene expression.
AUTHOR: Monia Brett P(a); Lesnik Elena A; Gonzalez Carolyn; Lima Walt F; McGee Danny; Guinosso Charles J; Kawasaki Andrew M; Cook P Dan; Freier Susan M
AUTHOR ADDRESS: (a)ISIS Pharm., 2280 Faraday Ave., Carlsbad, CA 92008**USA
JOURNAL: Journal of Biological Chemistry 268 (19):p14514-14522 1993
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We have used a previously described 17-mer phosphorothioate (Monia, B. P., Johnston, J. F., Ecker, D. J., Zounes, M. A., Lima, W. F., and Freier, S. M. (1992) J. Biol Chem. 267, 19954-19962) for structure-function analysis of 2'-sugar modifications including 2'-O-methyl, 2'-O-propyl, 2'-O-pentyl, and 2'-fluoro. These modifications were analyzed for hybridization affinity to complementary RNA and for antisense **activity** against the Ha-ras oncogene in cells using a highly sensitive transactivation reporter gene system. Hybridization analysis demonstrated that all of the 2'-**modified oligonucleotides** hybridized with greater affinity to RNA than an unmodified 2'-deoxy **oligonucleotide** with the rank order of affinity being 2'-fluoro gt 2'-O-methyl gt 2'-O-propyl gt 2'-O-pentyl gt 2'-deoxy. Evaluation of antisense **activities** of uniformly 2'-**modified oligonucleotides** revealed that these compounds were completely ineffective in inhibiting Haras gene expression. **Activity** was restored if the compound contained a stretch of at least five 2'-deoxy residues. This minimum deoxy length correlated perfectly with the minimum length required for efficient RNase H **activation** in vitro using partially purified mammalian RNase H enzyme. These chimeric 2'-**modified**/deoxy phosphorothioates displayed greater antisense potencies in inhibiting Ha-ras gene expression, compared with the unmodified uniform deoxy phosphorothioate. Furthermore, antisense potency correlated directly with affinity of a given 2' modification for its complementary RNA. These results demonstrate the importance of target affinity in the action of antisense **oligonucleotides** and of RNase H as a mechanism by which these compounds exert their effects.
? s s6 not s7

```
              46  S6
               7  S7
      S8      39  S6 NOT S7
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? t s8/3,ab/all

8/3,AB/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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12346912 BIOSIS NO.: 200000100414
Oligonucleotide conjugated to linear and branched high molecular weight polyethylene glycol as substrates for RNase H.
AUTHOR: Vorobjev P E(a); Zarytova V F(a); Bonora G M
AUTHOR ADDRESS: (a)Institute of Bioorganic Chemistry, Siberian Division of Russian Academy of Sciences, 630090, Novosibirsk**Russia

JOURNAL: Nucleosides & Nucleotides 18 (11-12):p2745-2750 Nov.-Dec., 1999
ISSN: 0732-8311
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Two conjugates of an anti-HIV **oligonucleotide** (ODN) with different high molecular weight monomethoxy polyethylene glycols (MPEGs) have been tested for their **activity** as substrate towards RNase H. The MPEG does not impede the formation of the regular hybrid duplex with the target RNA sequence as pointed out by the persistence of the RNase H **activity**; thus, these derivatives stimulate the hydrolysis of RNA by the enzyme at the same site and with the same extent of cleavage as the native sequence.

8/3,AB/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12329455 BIOSIS NO.: 200000082957
Similarities and differences in the RNase H **activities** of human immunodeficiency virus type 1 reverse transcriptase and moloney murine leukemia virus reverse transcriptase.
AUTHOR: Gao Hong-Qiang; Sarafianos Stefan G; Arnold Edward; Hughes Stephen H(a)
AUTHOR ADDRESS: (a)ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, MD, 21702-1201**USA
JOURNAL: Journal of Molecular Biology 294 (5):p1097-1113 Dec. 17, 1999
ISSN: 0022-2836
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Retroviral revXerse transcriptases (RTs) have an associated RNase H **activity** that can cleave RNA-DNA duplexes with considerable precision. We believe that the structure of the RNA-DNA duplexes in the context of RT determines the specificity of RNase H cleavage. To test this idea, we treated three related groups of synthetic RNA-DNA hybrids with either Moloney murine leukemia virus (MLV) RT or human immunodeficiency virus type 1 (HIV-1) RT. All of the hybrids were prepared using the same 81-base RNA template. The first series of RNase H substrates was prepared with complementary DNA **oligonucleotides** of different lengths, ranging from 6 to 20 nucleotides, all of which shared a common 5' end and were successively shorter at their 3' ends. The second series of **oligonucleotides** had a common 3' end but shorter 5' ends. The DNA **oligonucleotides** in the third series were all 20 bases long but had non-complementary stretches at either the 5' end, 3' end, or both ends. Several themes have emerged from the experiments with these RNA-DNA duplexes. (1) Both HIV-1 RT and MLV RT cleave fairly efficiently if the duplex region is at least eight bases long, but not if it is shorter. (2) Although, under the conditions we have used, both enzymes require the substrate to have a region of RNA-DNA duplex, both MLV RT and HIV-1 RT can cleave RNA outside the region that is part of the RNA-DNA duplex. (3) The polymerase domain of HIV-1 RT uses certain mismatched segments of RNA-DNA to position the enzyme for RNase H cleavage, whereas the polymerase domain of MLV RT does not use the same mismatched segments to define the position for RNase H cleavage. (4) For HIV-1 RT, a mismatched region near the RNase H domain can interfere with RNase H cleavage; cleavage is usually (but not always) more efficient if the mismatched segment is deleted. These results are discussed in regard to the structure of HIV-1 RT and the differences between HIV-1 RT and MLV RT.

8/3,AB/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12299511 BIOSIS NO.: 200000057378
Inhibition of plasminogen **activator** inhibitor release in endothelial cell cultures by antisense oligodeoxyribonucleotides with a 5'-end lipophilic modification.
AUTHOR: Kobylanska Anna; Pluskota Elzbieta; Swiatkowska Maria; Wojcik Marzena; Cierniewska-Cieslak Aleksandra; Krakowiak Agnieszka; Boczkowska Malgorzata; Pawlowska Zofia; Okruszek Andrzej(a); Koziolkiewicz Maria; Cierniewski Czeslaw S; Stec Wojciech J
AUTHOR ADDRESS: (a)Centre of Molecular and Macromolecular Studies, Department of Bioorganic Chemistry, Polish Academy of Sciences, H. Sienkiewicza 112, 90-363, Lodz**Poland
JOURNAL: Acta Biochimica Polonica 46 (3):p679-691 1999
ISSN: 0001-527X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: A series of conjugates containing residues of lipophilic alcohols covalently bound to 5' end of oligodeoxyribonucleotides targeted against human plasminogen **activator** inhibitor (PAI-1) mRNA was synthesized via the oxathiaphospholane approach. The highest anti-PAI-1 **activity** in EA.hy 926 endothelial cell cultures was found for conjugates containing menthyl or heptadecanyl groups linked with an **oligonucleotide** complementary to a segment of human PAI-1 mRNA. The phosphodiester antisense **oligonucleotides**, which otherwise exhibit only limited anti-PAI-1 **activity**, were found to be more **active** than phosphorothioate **oligonucleotides** when conjugated to lipophilic alcohol residues. For menthyl conjugates an evidence of antisense mechanism of inhibition was found.

8/3,AB/4 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12256999 BIOSIS NO.: 200000010501
Growth inhibition of cervical tumor cells by antisense oligodeoxynucleotides directed to the human papillomavirus type 16 E6 gene.
AUTHOR: Alvarez-Salas Luis M; Arpawong Thalida E; Dipaolo Joseph A(a)
AUTHOR ADDRESS: (a)Department of Health and Human Services, Laboratory of Biology, NCI/NIH, Building 37, Room 2A19, Bethesda, MD, 20892**USA
JOURNAL: Antisense & Nucleic Acid Drug Development 9 (5):p441-450 Oct., 1999
ISSN: 1087-2906
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Human papillomavirus type 16 (HPV-16) is the HPV type most frequently associated with cervical carcinomas. Based on our previous research with anti-HPV ribozymes, we developed a 16-nucleotide antisense oligodeoxynucleotide (AntiE6) able to direct RNase H **activity** on full-length HPV-16 E6/E7 mRNA. Although the precise mechanism is not completely understood, addition of 50 muM AntiE6 oligodeoxynucleotide in sterile water caused a significant decrease in the growth rate of CaSki and QGU cervical tumor cell lines. In contrast, addition of a mismatched

mutant oligodeoxynucleotide (M7) did not affect cell growth after 72 hours. Treatment with AntiE6 resulted in down-regulation of E6/E7 mRNA and an increase in p53 levels in QGU cells. AntiE6 was also able to (>70%) inhibit significantly growth of transplanted cervical tumors in nude mice after 2 weeks treatment using constant delivery by osmotic pumps. These results indicate that the AntiE6 antisense oligodeoxynucleotides can act as a therap eutic agent against cervical carcinomas.

8/3,AB/5 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12187896 BIOSIS NO.: 199900482745
Translation arrest by RNase H incompetent antisense **oligonucleotides**.

AUTHOR: Robbins I; Gee J E; Mitta G; Rayner B; Vichier-Guerre S; Leng M; Van der Laan A; van Boom J; Nelson J S; Lebleu B(a)
AUTHOR ADDRESS: (a)Institut de Genetique Moleculaire, CNRS, Montpellier** France
JOURNAL: Nucleosides & Nucleotides 18 (6-7):p1667-1668 June-July, 1999
ISSN: 0732-8311
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Most second-generation ON antisense analogs do not **activate** RNase H. Alternative strategies to arrest translation in a reticulocyte cell-free assay programmed by VSV mRNAs have been explored.

8/3,AB/6 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12154809 BIOSIS NO.: 199900449658
Molecular mechanisms of antisense drugs: Human RNase H.
AUTHOR: Crooke Stanley T(a)
AUTHOR ADDRESS: (a)Isis Pharmaceuticals, Inc., 2292 Faraday Avenue, Carlsbad, CA, 92008**USA
JOURNAL: Antisense & Nucleic Acid Drug Development 9 (4):p377-379 Aug., 1999
ISSN: 1087-2906
DOCUMENT TYPE: Article
RECORD TYPE: Citation
LANGUAGE: English

8/3,AB/7 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12128325 BIOSIS NO.: 199900423174
Peptide-**oligonucleotide** phosphorothioate conjugates with membrane translocation and nuclear localization properties.
AUTHOR: Antopolsky Maxim; Azhayeva Elena; Tengvall Unni; Auriola Seppo; Jaeaeskelaeinen Ilpo; Roenkkoe Seppo; Honkakoski Paavo; Urtti Arto; Loennberg Harri; Azhayev Alex(a)
AUTHOR ADDRESS: (a)Department of Pharmaceutical Chemistry, University of Kuopio, FIN-70211, Kuopio**Finland
JOURNAL: Bioconjugate Chemistry 10 (4):p598-606 July-Aug., 1999
ISSN: 1043-1802
DOCUMENT TYPE: Article

RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Eighteen peptide-**oligonucleotide** phosphorothioate conjugates were prepared in good yield and thoroughly characterized with electrospray ionization mass spectra. When applied to the living cells, conjugates exhibiting membrane translocation and nuclear localization properties displayed efficient intracellular penetration but failed to show any serious antisense effect. Studies on the intracellular distribution of the fluorescein-labeled conjugates revealed their trapping in endosomes.

8/3,AB/8 (Item 8 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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12030265 BIOSIS NO.: 199900310784
DNA aptamers selected against the HIV-1 trans-**activation**-responsive RNA element form RNA-DNA kissing complexes.
AUTHOR: Boiziau Claudine; Dausse Eric; Yurchenko Ludmila; Toulme Jean-Jacques(a)
AUTHOR ADDRESS: (a)IFR Pathologies Infectieuses, INSERM U 386, Universite Victor Segalen, 146 rue Leo Saignat, F-33**France
JOURNAL: Journal of Biological Chemistry 274 (18):p12730-12737 April 30, 1999
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: In vitro selection was performed in a DNA library, made of **oligonucleotides** with a 30-nucleotide random sequence, to identify ligands of the human immunodeficiency virus type-1 trans-**activation** -responsive (TAR) RNA element. Aptamers, extracted after 15 rounds of selection-amplification, either from a classical library of sequences or from virtual combinatorial libraries, displayed an imperfect stem-loop structure and presented a consensus motif 5' ACTCCCAT in the apical loop. The six central bases of the consensus were complementary to the TAR apical region, giving rise to the formation of RNA-DNA kissing complexes, without disrupting the secondary structure of TAR. The RNA-DNA kissing complex was a poor substrate for Escherichia coli RNase H, likely due to steric and conformational constraints of the DNA/RNA heteroduplex. 2'-O-Methyl derivatives of a selected aptamer were binders of lower efficiency than the parent aptamer in contrast to regular sense/anti-sense hybrids, indicating that the RNA/DNA loop-loop region adopted a non-canonical heteroduplex structure. These results, which allowed the identification of a new type of complex, DNA-RNA kissing complex, demonstrate the interest of in vitro selection for identifying non-antisense **oligonucleotide** ligands of RNA structures that are of potential value for artificially modulating gene expression.

8/3,AB/9 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11872428 BIOSIS NO.: 199900118537
Structure and function of a small RNA that selectively inhibits internal ribosome entry site-mediated translation.
AUTHOR: Venkatesan Arun; Das Saumitra; Dasgupta Asim(a)
AUTHOR ADDRESS: (a)Mol. Biol. Inst., Univ. Calif., Los Angeles, CA 90095-1747**USA

JOURNAL: Nucleic Acids Research 27 (2):p562-572 Jan. 15, 1999
ISSN: 0305-1048
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A 60 nt long RNA termed IRNA, isolated from the yeast Saccharomyces cerevesiae, was previously shown to selectively block internal ribosome entry site (IRES)-mediated translation without interfering with cap-dependent translation of cellular mRNAs both in vivo and in vitro. IRNA specifically bound cellular proteins believed to be important for IRES-mediated translation. We demonstrate here that a complementary copy of IRNA (cIRNA) is also **active** in blocking IRES-mediated translation and that it binds many of the same cellular proteins that IRNA does. We have probed the secondary structure of both IRNA and cIRNA using single-strand- and double-strand-specific nucleases as well as using **oligonucleotide** hybridization followed by RNase H digestion. Both IRNA and cIRNA share secondary structural homology, although distinct differences do exist between the two structures. Mutational analysis of IRNA shows that sequences that form both the main stem and one loop are critical for its translation inhibitory **activity**. Maintenance of the established secondary structure appears to be required for both IRNA's ability to bind cellular trans-acting proteins believed to be required for IRES-mediated translation and its ability to block IRES-mediated translation.

8/3,AB/10 (Item 10 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11811671 BIOSIS NO.: 199900057780
Sequence-specific RNase H cleavage of gag mRNA from HIV-1 infected cells by an antisense **oligonucleotide** in vitro.
AUTHOR: Veal Gareth J(a); Agrawal Sudhir; Byrn Randal A
AUTHOR ADDRESS: (a)Cancer Res. Unit, Med. Sch., Framlington Place, Univ. Newcastle, Newcastle Upon Tyne NE2 4HH**UK
JOURNAL: Nucleic Acids Research 26 (24):p5670-5675 Dec. 15, 1998
ISSN: 0305-1048
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We have used a ribonuclease protection assay to investigate RNase H cleavage of HIV-1 mRNA mediated by phosphorothioate antisense **oligonucleotides** complementary to the gag region of the HIV-1 genome in vitro. Cell lysate experiments in H9 and U937 cells chronically infected with HIV-1 IIIB showed RNase H cleavage of unspliced gag message but no cleavage of spliced message which did not contain the target gag region. RNase H cleavage products were detected at **oligonucleotide** concentrations as low as 0.01 muM and the RNase H **activity** was seen to be concentration dependent. Similar experiments with 1-, 3- and 5-mismatch **oligonucleotides** demonstrated sequence specificity at low concentrations, with cleavage of gag mRNA correlating with the predicted **activities** of the parent and mismatch **oligonucleotides** based on their hybridization melting temperatures. Experiments in living cells suggested that RNase H-specific antisense **activity** was largely determined by the amount of **oligonucleotide** taken up by the different cell lines studied. RNase H cleavage products were detected in antisense **oligonucleotide** treated MT-4 cells acutely infected with HIV-1 IIIB, but not in infected H9 cells treated with **oligonucleotide** under the same conditions. The data presented demonstrate potent and specific RNase H cleavage of HIV-1 mRNA mediated by an antisense **oligonucleotide** targeted to HIV-1 gag mRNA, and are in agreement with previous reports that the major

obstacle to demonstrating antisense **activity** in living cells remains the lack of penetration of these agents into the desired cellular compartment.

8/3,AB/11 (Item 11 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11767281 BIOSIS NO.: 199900013390
Modification of phosphorothioate **oligonucleotides** yields potent analogs with minimal toxicity for antisense experiments in the CNS.
AUTHOR: Ho Siew Peng(a); Livanov Valentin; Zhang Wei; Li Jing-Hua; Lesher Treena
AUTHOR ADDRESS: (a)CNS Dep., DuPont Pharmaceuticals Research Laboratories, P.O. Box 80400 Experimental Station, Wil**USA
JOURNAL: Molecular Brain Research 62 (1):p1-11 Nov. 12, 1998
ISSN: 0169-328X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: There is increasing evidence that phosphorothioate **oligonucleotides** infused into the brain can cause a host of undesired side effects which compromise the antisense experiment. In studies on the corticotropin releasing factor type-2 receptor, several phosphorothioate **oligonucleotides** administered intraventricularly produced significant weight loss in rats. Four different phosphodiester and phosphorothioate **oligonucleotide** analogs were examined to identify molecules which could eliminate these side effects while maintaining good potency for antisense inhibition. Of these, chimeric **oligonucleotides** consisting of a mixed phosphodiester-phosphorothioate backbone, and having 2'-methoxyribonucleotide modifications in 60% of the **oligonucleotide** were the most optimal. Rats treated with these chimeric **oligonucleotides** gained weight at rates identical to that of saline-treated controls. In addition, the antisense **oligonucleotide** but not the mismatch control sequence reduced corticotropin releasing factor type-2 receptor binding of 125iodo-sauvagine in the lateral septum by 40-60% after 5 daily injections. Increasing the dosing period to 9 days reduced receptor binding by 78%. Reductions in protein binding were accompanied by comparable reductions in the in situ hybridization signal of the corticotropin releasing factor type-2 receptor mRNA. However, when an **oligonucleotide** analog incapable of supporting ribonuclease H **activity** was used, neither protein nor RNA binding levels were changed compared to saline-treated controls. These results suggest that ribonuclease H or enzymes with similar **activity** are critical to the antisense inhibition observed in the lateral septum.

8/3,AB/12 (Item 12 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11485044 BIOSIS NO.: 199800266376
Assessment of high-affinity hybridization, RNase H cleavage, and covalent linkage in translation arrest by antisense **oligonucleotides**.
AUTHOR: Gee Jay E; Robbins Ian; Van Der Laan Alexander C; Van Boom Jacques H; Colombier Caroline; Leng Marc; Raible Annette M; Nelson Jeffrey S; Lebleu Bernard(a)
AUTHOR ADDRESS: (a)Int. Genet. Mol. Montpellier, CNRS, 1919 route de Mende, F-34293 Montpellier Cedex 5**France
JOURNAL: Antisense & Nucleic Acid Drug Development 8 (2):p103-111 April, 1998

ISSN: 1087-2906
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Antisense **oligonucleotides** (ONs) are designed to hybridize target mRNA in a sequence-specific manner and inhibit gene expression by preventing translation, either by **activation** of RNase H or steric blockage of the ribosome complex. Second-generation ONs, which possess greater binding affinity for target RNA relative to the isosequential phosphodiester (PO) ONs, have been developed and include, among others, peptide nucleic acids (PNA) and N3' fwdarw P5' phosphoramidate **oligonucleotides** (npONs). In the present study, PNA and npON derivatives were targeted to the coding portion of the complementary mRNA of the N protein of the vesicular stomatitis virus (VSV) in order to evaluate their ability to arrest translation in an in vitro rabbit reticulocyte lysate system. High-affinity hybridization of ONs lacking RNase H **activity** was not sufficient to block translation in this test system. Only antisense ONs acting via an RNase H mechanism or by steric hindrance through covalent attachment (via transplatin modification) to the target mRNA were found to definitively arrest translation in this study.

8/3,AB/13 (Item 13 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11448732 BIOSIS NO.: 199800230064
Characterization of RNA strand displacement synthesis by Moloney murine leukemia virus reverse transcriptase.
AUTHOR: Kelleher Colleen D; Champoux James J(a)
AUTHOR ADDRESS: (a)Dep. Microbiol., Box 357242, Sch. Med., Univ. Washington, Seattle, WA 98195-7242**USA
JOURNAL: Journal of Biological Chemistry 273 (16):p9976-9986 April 17, 1998
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The RNase H **activity** of reverse transcriptase (RT) is presumably required to cleave the RNA genome following minus strand synthesis to free the DNA for use as a template during plus strand synthesis. However, since RNA degradation by RNase H appears to generate RNA fragments too large to spontaneously dissociate from the minus strand, we have investigated the possibility that RNA displacement by RT during plus strand synthesis contributes to the removal of RNA fragments. By using an RNase H!- mutant of Moloney murine leukemia virus (M-MuLV) RT, we demonstrate that the polymerase can displace long regions of RNA in hybrid duplex with DNA but that this **activity** is approximately 5-fold slower than DNA displacement and 20-fold slower than non-displacement synthesis. Furthermore, we find that although certain hybrid sequences seem nearly refractory to the initiation of RNA displacement, the same sequences may not significantly impede synthesis when preceded by a single-stranded gap. We find that the rate of RNA displacement synthesis by wild-type M-MuLV RT is significantly greater than that of the RNase H!- RT but remains less than the rate of non-displacement synthesis. M-MuLV nucleocapsid protein increases the rates of RNA and DNA displacement synthesis approximately 2-fold, and this **activity** appears to require the zinc finger domain.

8/3,AB/14 (Item 14 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11246609 BIOSIS NO.: 199800027941
Anti-influenza virus **activities** of nicked and circular dumbbell RNA/DNA chimeric **oligonucleotides**.
AUTHOR: Yanakawa Hidefumi; Ishibashi Toshiaki; Abe Takayu; Hatta Toshifumi; Takai Kazuyuki; Takaku Hiroshi
AUTHOR ADDRESS: Dep. Ind. Chem., Chiba Inst. Technol., Tsudanuma, Narashino, Chiba 275**Japan
JOURNAL: Nucleosides & Nucleotides 16 (7-9):p1713-1716 July-Sept., 1997
ISSN: 0732-8311
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We have designed a new type of antisense **oligonucleotide**, containing two hairpin loop structures with RNA/DNA base pairs (sense (RNA) and antisense (DNA)) in the double helical stem (nicked and circular dumbbell DNA/RNA chimeric **oligonucleotides**). The reaction of the nicked and circular dumbbell DNA/RNA chimeric **oligonucleotides** with RNase H gave the corresponding anti-DNA together with the sense RNA cleavage products. These **oligonucleotides** were more resistant to exonuclease attack. We also describe the anti-Fluv **activities** of nicked and circular dumbbell DNA/RNA chimeric **oligonucleotides**.

8/3,AB/15 (Item 15 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11223187 BIOSIS NO.: 199800004519
The 5'-exonuclease **activity** of bacteriophage T4 RNase H is stimulated by the T4 gene 32 single-stranded DNA-binding protein, but its flap endonuclease is inhibited.
AUTHOR: Bhagwat Medha; Hobbs Lisa J; Nossal Nancy G(a)
AUTHOR ADDRESS: (a)Lab. Molecular Cellular Biol., NIDDK, Natl. Inst. Health, Build. 8, Room 2A-19, Bethesda, MD 208**USA
JOURNAL: Journal of Biological Chemistry 272 (45):p28523-28530 Nov. 7, 1997
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Bacteriophage T4 RNase H is a 5'- to 3'-nuclease that has exonuclease **activity** on RNAcntdotDNA and DNAcntdotDNA duplexes and can remove the pentamer RNA primers made by the T4 primase-helicase (Hollingsworth, H. C., and Nossal, N. G. (1991) J. Biol. Chem. 266,1888-1897; Hobbs, L. J., and Nossal, N. G. (1996) J. Bacteriol. 178, 6772-6777). Here we show that this exonuclease degrades duplex DNA nonprocessively, releasing a single **oligonucleotide** (nucleotides 1-4) with each interaction with the substrate. Degradation continues nonprocessively until the enzyme stops 8-11 nucleotides from the 3'-end of the substrate. T4 gene 32 single-stranded DNA-binding protein strongly stimulates the exonuclease **activity** of T4 RNase H, converting it into a processive nuclease that removes multiple short **oligonucleotides** with a combined length of 10-50 nucleotides each time it binds to the duplex substrate. 32 protein must bind on single-stranded DNA behind T4 RNase H for processive degradation. T4 RNase H also has a flap endonuclease **activity** that cuts preferentially on either side of the junction between single- and double-stranded DNA in flap and fork DNA structures. In contrast to the exonuclease, the endonuclease is inhibited completely by 32 protein binding to the single strand of the flap substrate. These results suggest

an important role for T4 32 protein in controlling T4 RNase H degradation of RNA primers and adjacent DNA during each lagging strand cycle.

8/3,AB/16 (Item 16 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11191609 BIOSIS NO.: 199799812754
Effects of 5-(N-aminohexyl)carbamoyl-2'-deoxyuridine on endonuclease stability and the ability of oligodeoxynucleotide to **activate** RNase H.
AUTHOR: Ueno Yoshihito; Kumagai Izumi; Haginoya Noriyasu; Matsuda Akira(a)
AUTHOR ADDRESS: (a)Fac. Pharmaceutical Sci., Hokkaido Univ., Kita-12, Nishi-6, Kita-ku, Sapporo 060**Japan
JOURNAL: Nucleic Acids Research 25 (19):p3777-3782 1997
ISSN: 0305-1048
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: To evaluate an endonuclease resistance property of oligodeoxynucleotides (ODNs) containing 5-(N-aminohexyl)carbamoyl-2'-deoxyuridines (Hs) and to elucidate whether a duplex consisting of the ODN analogue and its complementary RNA induces RNase H **activity**, the ODNs containing the deoxyuridine analogues, Hs, at intervals of one, two, three, four and five natural nucleosides were synthesized. From partial hydrolysis of these ODNs with nuclease S1 (an endonuclease), it was found that the ODNs became more stable towards nucleolytic hydrolysis by the enzyme as the number of H increased. Furthermore, to examine whether the duplexes composed of the ODNs containing Hs and their complementary RNAs are substrates for RNase H or not, the duplexes of these ODNs and their complementary RNA strands were treated with Escherichia coli RNase H. It was found that cleavage of the RNA strands by the enzyme was kinetically affected by the introduction of Hs into the duplexes.

8/3,AB/17 (Item 17 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11131360 BIOSIS NO.: 199799752505
Transfer of Tat and release of TAR RNA during the **activation** of the human immunodeficiency virus type-1 transcription elongation complex.
AUTHOR: Keen Nicholas J; Churcher Mark J; Karn Jonathan(a)
AUTHOR ADDRESS: (a)MRC Lab. Molecular Biol., Hills Road, Cambridge CB2 2QH **UK
JOURNAL: EMBO (European Molecular Biology Organization) Journal 16 (17):p5260-5272 1997
ISSN: 0261-4189
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The HIV-1 trans-**activator** protein, Tat, is a potent **activator** of transcriptional elongation. Tat is recruited to the elongating RNA polymerase during its transit through the trans-**activation** response region (TAR) because of its ability to bind directly to TAR RNA expressed on the nascent RNA chain. We have shown that transcription complexes that have acquired Tat produce 3-fold more full-length transcripts than complexes not exposed to Tat. Western blotting experiments demonstrated that Tat is tightly associated with the paused polymerases. To determine whether TAR RNA also becomes attached to the transcription complex, DNA **oligonucleotides** were annealed to the nascent chains on the arrested complexes and the RNA was cleaved by RNase H. After cleavage, the 5' end of the nascent chain, carrying TAR

RNA, is quantitatively removed, but the 3' end of the transcript remains associated with the transcription complex. Even after removal of TAR RNA, transcription complexes that have been **activated** by Tat show enhanced processivity. We conclude that Tat, together with cellular co-factors, becomes attached to the transcription complex and stimulates processivity, whereas TAR RNA does not play a direct role in the **activation** of elongation and is used simply to recruit Tat and cellular co-factors.

8/3,AB/18 (Item 18 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11099055 BIOSIS NO.: 199799720200
Binding of small dumbbell **oligonucleotides** to MOMULV reverse transcriptase: Inhibitory properties of RNase H **activity**.
AUTHOR: Kumar Ajay(a); Moreau Serge; Toulme Jean-Jacques
AUTHOR ADDRESS: (a)NII, New Delhi**India
JOURNAL: FASEB Journal 11 (9):pA1154 1997
CONFERENCE/MEETING: 17th International Congress of Biochemistry and Molecular Biology in conjunction with the Annual Meeting of the American Society for Biochemistry and Molecular Biology San Francisco, California, USA August 24-29, 1997
ISSN: 0892-6638
RECORD TYPE: Citation
LANGUAGE: English

8/3,AB/19 (Item 19 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10943061 BIOSIS NO.: 199799564206
Alanine-scanning mutations in the "Primer grip" of p66 HIV-1 reverse transcriptase result in selective loss of RNA priming **activity**.
AUTHOR: Powell Michael D; Ghosh Madhumita; Jacques Pamela S; Howard Kathryn J; Le Grice Stuart F J; Levin Judith G(a)
AUTHOR ADDRESS: (a)Lab. Molecular Genetics, NICHD, Build. 6B, Room 216, Natl. Inst. Health, Bethesda, MD 20892**USA
JOURNAL: Journal of Biological Chemistry 272 (20):p13262-13269 1997
ISSN: 0021-9258
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Alanine-scanning mutants of the primer grip region of human immunodeficiency virus type 1 reverse transcriptase were tested for their ability to extend RNA and DNA versions of the polypurine tract primer, and an **oligonucleotide** representing the 18-nucleotide sequence at the 3' end of tRNA-Lys3. A majority of the mutant enzymes were either completely or severely deficient in RNA priming **activity**, but, with only one exception, were able to efficiently extend DNA versions of the same primers. The mutant enzymes were able to bind to RNA primers, indicating that the defect in RNA priming was not simply a loss of binding **activity**. Mutations at positions 229, 233, and 235 dramatically reduced the amount of specific RNase H cleavage at the 3' terminus of the polypurine tract, which is required for primer removal. An alanine substitution at position 232 led to loss of cleavage specificity, although total **activity** was close to the wild-type level. Taken together, these results demonstrate for the first time that there are residues in human immunodeficiency virus type 1 reverse transcriptase which are specifically involved in protein-nucleic acid interactions with RNA primers.

8/3,AB/20 (Item 20 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10917259 BIOSIS NO.: 199799538404
Anti-influenza virus **activity** by circular dumbbell RNA/DNA chimeric oligo-nucleotides.
AUTHOR: Yamakawa H; Ishibashi T; Abe T; Hatta T; Takai K; Takaku H
AUTHOR ADDRESS: Chiba Inst. Technol., Tsudanuma, Narashino, Chiba 275** Japan
JOURNAL: Antiviral Research 34 (2):pA87 1997
CONFERENCE/MEETING: Meeting of the International Society for Antiviral Research and the Tenth International Conference on Antiviral Research Atlanta, Georgia, USA April 6-11, 1997
ISSN: 0166-3542
RECORD TYPE: Citation
LANGUAGE: English

8/3,AB/21 (Item 21 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10757995 BIOSIS NO.: 199799379140
Inhibition of human TNF-alpha and LT in cell-free extracts and in cell culture by antisense **oligonucleotides**.
AUTHOR: Lefebvre D'Hellencourt Christian(a); Diaw Lena; Cornillet Pascale; Guenounou Moncef
AUTHOR ADDRESS: (a)INSERM U298, CHU, 4 rue Larrey, 49033 Angers Cedex 01** France
JOURNAL: Biochimica et Biophysica Acta 1317 (3):p168-174 1996
ISSN: 0006-3002
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Antisense **oligonucleotides** (ODN), complementary to mRNA of human tumor necrosis factor alpha (TNF-alpha) and lymphotoxin (LT) were tested for their ability to inhibit TNFs. TNFs production was studied in cell-free systems including wheat germ extract (WGE) and rabbit reticulocyte lysate (RRL). All ODN were effective in WGE at low concentration (0.2 mu-M), except those targeted to the 3' region of TNF-alpha mRNA. A short ODN complementary to a common region between TNF-alpha and LT inhibited both TNFs. In contrast, high ODN concentration (50 mu-M) was needed to inhibit LT mRNA translation in RRL, whereas no clear inhibition of TNF-alpha was observed unless RNase H was added to the translation mixture. ODN effects on TNFs production by stimulated cell tine in culture were also investigated. Three ODN - one located in the 5'-untranslated region, one spanning the AUG initiation codon and one downstream of this AUG were the most effective sequences to decrease TNF-alpha production. Two ODN targeted to the AUG initiation codon of LT were also able to inhibit its production. In conclusion we confirm the role of RNase H in cell free systems, and we found that there is no correlation between ODN efficiency in a cell-free system nor in cell culture. Efficient ODN could be used for in vitro investigation of the role of TNF-alpha and LT in mechanism in which they are involved.

8/3,AB/22 (Item 22 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10751845 BIOSIS NO.: 199799372990
Combinatorial screening and rational optimization for hybridization to folded hepatitis C virus RNA of **oligonucleotides** with biological antisense **activity**.

AUTHOR: Lima Walt F; Brown-Driver Vickie; Fox Maureen; Hanecak Ronnie; Bruice Thomas W(a)
AUTHOR ADDRESS: (a)Dep. Res. Medicinal Chem., Isis Pharm., 2292 Faraday Ave., Carlsbad, CA 92008**USA
JOURNAL: Journal of Biological Chemistry 272 (1):p626-638 1997
ISSN: 0021-9258
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We describe our initial application of a biochemical strategy, comprising combinatorial screening and rational optimization, which directly identifies **oligonucleotides** with maximum affinity (per unit length), specificity, and rates of hybridization to structurally preferred sites on folded RNA, to the problem of design of antisense **oligonucleotides** **active** against the hepatitis C virus (HCV). A fully randomized sequence DNA **oligonucleotide** (10-mer) library was equilibrated with each of two folded RNA fragments (200 and 370 nucleotides (nt)), together spanning the 5' 440 nt of an HCV transcript (by overlapping 130 nt), which were varied over a range of concentrations. The equilibrations were performed in solution under conditions determined to preserve RNA structure and to limit all RNA-DNA library **oligonucleotide** interactions to 1:1 stoichiometry. Subsequent Escherichia coli RNase H (endoribonuclease H: EC 3.1.26.4) cleavage analysis identified two preferred sites of highest affinity heteroduplex hybridization. The lengths and sequences of different substitute chemistry **oligonucleotides** complementary to these sites were rationally optimized using an iterative and quantitative analysis of binding affinity and specificity. Thus, DNA **oligonucleotides** that hybridized with the same affinity to the preferred sites in the folded RNA fragments found by screening as to short (ltoreq -25 nt) RNA complements were identified but were found to vary in length (10-18 nt) from site to site. Phosphorothioate (P=S) and 2'-fluoro (2'-F) uniformly substituted **oligonucleotides** also were found, which hybridized optimally to these sites, supporting the design of short (10-15-nt) and maximally specific **oligonucleotides** that are more nuclease-resistant (via P=S) and have higher affinity (via 2'-F) than DNA. Finally, the affinities of DNA and uniform 2'-F-, P=S-substituted 10-20-mer **oligonucleotide** complements for the best hybridization site, from HCV nt 355 to nt 364-374, closely corresponded to antisense mechanism inhibition **activities** in an in vitro translation assay and in a human cell-based HCV core protein expression assay, respectively. These results validate our strategy for the selection of hybridization-optimized and biologically **active** antisense **oligonucleotides** targeting HCV RNA and support the potential for utility in further applications.

8/3,AB/23 (Item 23 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10428524 BIOSIS NO.: 199699049669
Potent antisense **oligonucleotides** to the human multidrug resistance-1 mRNA are rationally selected by mapping RNA-accessible sites with **oligonucleotide** libraries.
AUTHOR: Ho Siew Peng(a); Britton Dustin H O; Stone Barry A; Behrens Davette L; Leffet Lynn M; Hobbs Frank W; Miller Jeff A; Trainor George L
AUTHOR ADDRESS: (a)DuPont Merck Res. Lab., P.O. Box 80400, Experimental Station, Wilmington, DE 19880-0400**USA
JOURNAL: Nucleic Acids Research 24 (10):p1901-1907 1996
ISSN: 0305-1048
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Antisense **oligonucleotides** can vary significantly and unpredictably in their ability to inhibit protein synthesis. Libraries of chimeric **oligonucleotides** and RNase H were used to cleave and thereby locate sites on human multidrug resistance-1 RNA transcripts that are relatively accessible to **oligonucleotide** hybridization. In cell culture, antisense sequences designed to target these sites were significantly more **active** than **oligonucleotides** selected at random. This methodology should be generally useful for identification of potent antisense sequences. Correlation between **oligonucleotide** **activity** in the cell culture assay and in an in vitro RNase H assay supports the proposed role of the enzyme in the mechanism of antisense suppression in the cell.

8/3,AB/24 (Item 24 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10357710 BIOSIS NO.: 199698812628
Protein kinase A-directed antisense restrains tumor growth: Use of phosphorothioate **oligonucleotides** containing segments of either 2'-O-methyl-oligoribonucleotide or methylphosphate **oligonucleotide**.

AUTHOR: Cho-Chung Y S(a); Nesterova M(a); Agrawal S; Noguchi K
AUTHOR ADDRESS: (a)National Cancer Inst., Bethesda, MD 20892**USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 37 (0):p351 1996
CONFERENCE/MEETING: 87th Annual Meeting of the American Association for Cancer Research Washington, D.C., USA April 20-24, 1996
ISSN: 0197-016X
RECORD TYPE: Citation
LANGUAGE: English

8/3,AB/25 (Item 25 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10317682 BIOSIS NO.: 199698772600
Properties and anti-HIV **activating** of hairpin antisense **oligonucleotides** containing 2'-methoxynucleosides with base-pairing in the stem region at the 3'-end.
AUTHOR: Hosono K; Kuwasaki T; Inagawa T; Takai K; Nakashima H; Saito T; Yamamoto N; Takaku H(a)
AUTHOR ADDRESS: (a)Dep. Industrial Chem., Chiba Inst. Technol., Narashino, Chiba 275**Japan
JOURNAL: Antiviral Chemistry & Chemotherapy 7 (2):p86-93 1996
ISSN: 0956-3202
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A new type of hairpin antisense oligodeoxyribonucleotide, containing 2'-methoxynucleosides with base-pairing in the stem region at the 3'-end, was tested for 3'-exonuclease resistance and anti-HIV **activity**. An increased resistance to nuclease degradation has been observed by incubation of the hairpin oligo-nucleotides with DNA polymerase and foetal bovine serum. Of particular interest is the hairpin antisense **oligonucleotide** containing 2'methoxynucleosides with base-pairing in the stem region at the 3'-end, which has increased nuclease resistance and hybridizes effectively with a complementary RNA. Furthermore, these compounds were assayed for inhibition of virus replication in HIV-1 infected MT-4 cells. In the anti-HIV **activity** test, the hairpin **oligonucleotide** phosphorothioate derivatives showed higher anti-HIV **activities** compared to their linear

counterparts.

8/3,AB/26 (Item 26 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10272442 BIOSIS NO.: 199698727360
Cycling probe technology with RNase H attached to an **oligonucleotide**.

AUTHOR: Bekkaoui F(a); Poisson I; Crosby W; Cloney L; Duck P
AUTHOR ADDRESS: (a)ID Biomedical Corp., 8855 Northbrook Court, Burnaby, BC V5J 5J1**Canada
JOURNAL: Biotechniques 20 (2):p240-244, 246-248 1996
ISSN: 0736-6205
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A streptavidin-RNase H gene fusion was constructed by cloning the Thermus thermophilus RNase H coding sequence in the streptavidin expression vector pTSA18F. The gene was expressed in Escherichia coli, and the resulting fusion protein was purified to apparent homogeneity. The fusion protein was shown to have a molecular weight of 128 kDa and to consist of four subunits. Furthermore, heat treatment of the fusion enzyme showed that it was stable as a tetramer at 65 degree C. The fusion enzyme was shown to have both biotin binding and RNase H catalytic properties. Using cycling probe technology (CPT), the fusion enzyme was compared to the native RNase H with a biotinylated probe at different ratios of probe:enzyme and varying amounts of synthetic target DNA. At a ratio of 1:1, the fusion enzyme was **active** in CPT, but the native enzyme was not; both enzymes were **active** at a 1:5000 ratio of probe:enzyme. The fusion enzyme was further tested using biotinylated and non-biotinylated probes and was shown to be **active** at a 1:1 ratio with the biotinylated probe but not with the non-biotinylated probe. These experiments show that through binding of the streptavidin-RNase H fusion enzyme to the biotinylated probe, the efficiency of the cycling probe reaction is enhanced.

8/3,AB/27 (Item 27 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10219167 BIOSIS NO.: 199698674085
Stereodifferentiation. The effect of P chirality of oligo(nucleoside phosphorothioates) on the **activity** of bacterial RNase H.
AUTHOR: Koziolkiewicz Maria; Krakowiak Agnieszka; Kwinkowski Marek; Boczkowska Malgorzata; Stec Wojciech J(a)
AUTHOR ADDRESS: (a)Polish Acad. Sci., Cent. Mol. Macromol. Studies, Dep. Bioorganic Chem., Sienkiewicza 112, 90-363**Poland
JOURNAL: Nucleic Acids Research 23 (24):p5000-5005 1995
ISSN: 0305-1048
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: P stereoregular phosphorothioate analogs of pentadecamer 5'-d(AGATGTTTGAGCTCT)-3' were synthesized by the oxathiaphospholane method. Their diastereomeric purity was assigned by means of enzymatic degradation with nuclease P1 and, independently, with snake venom phosphodiesterase. DNA-RNA hybrids formed by phosphorothioate **oligonucleotides** (PS-oligos) with the corresponding complementary pentadecaribonucleotide were treated with bacterial RNase H. The DNA-RNA complex containing the PS-oligo of (all-R-P) configuration was found to

be more susceptible to RNase H-dependent degradation of the pentadecaribonucleotide compared with hybrids containing either the (all-S-P) counterpart or the so called 'random mixture of diastereomers' of the pentadeca(nucleoside phosphorothioate). This stereodependence of RNase H action was also observed for a polyribonucleotide (475 nt) hybridized with these phosphorothioate **oligonucleotides**. The results of melting studies of PS-oligo-RNA hybrids allowed a rationalization of the observed stereodifferentiation in terms of the higher stability of heterodimers formed between oligoribonucleotides and (all-R-P)-oligo(nucleoside phosphorothioates), compared with the less stable heterodimers formed with (all-S-P)-oligo(nucleoside phosphorothioates) or the random mixture of diastereomers.

8/3,AB/28 (Item 28 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

09973893 BIOSIS NO.: 199598428811
Analysis of a ribonuclease H digestion of N3' fwdarw P5' phosphoramidate-RNA duplexes by capillary gel electrophoresis.
AUTHOR: Dedionisio Lawrence(a); Gryaznov Sergei M
AUTHOR ADDRESS: (a)Lynx Therapeutics Incorporated, 3832 Bay Center Place, Hayward, CA 94545**USA
JOURNAL: Journal of Chromatography B Biomedical Applications 669 (1):p 125-131 1995
ISSN: 0378-4347
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Phosphodiester **oligonucleotides** (ODNs) and their analogs are presently being investigated as potential antisense therapeutics in the treatment of viral infections and various forms of cancer. Here, we would like to report results from an investigation of **activity** for a ribonuclease H (RNase H) mediated RNA digestion assay in the duplexes formed by an ODN or the ODN analog, N3' fwdarw P5' phosphoramidate (3'-phosphoramidate), and complimentary RNA strands. Capillary gel electrophoresis (CGE) proved to be an effective method for determining RNA hydrolysis in the presence of RNase H. RNA and an ODN or RNA and a 3'-phosphoramidate were hybridized in a Tris-HCl, MgCl-2 buffer at room temperature (RT) and incubated with RNase H. Digestions were carried out at RT or at 37 degree C. Control samples were unhybridized RNA with RNase H, RNA without RNase H, and duplexes (RNA-ODN or 3'-phosphoramidate) without RNase H. All controls were incubated in Tris-HCl, MgCl-2 buffer, and sample aliquots were analyzed at various time intervals. A homodecamer, (dT)-10, was used as an internal standard to determine the relative migration time of the RNA strand. The final digestion products for the duplexes and the various controls were monitored by CGE. In addition, polyacrylamide gel electrophoresis (PAGE) was used in conjunction with Stains-All (staining) and a densitometric analysis to verify CGE results.

8/3,AB/29 (Item 29 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

09923382 BIOSIS NO.: 199598378300
Phosphorothioate **oligonucleotides** block reverse transcription by the RNase H **activity** associated with the HIV-1 polymerase.
AUTHOR: Hatta Toshifumi; Takai Kazuyuki; Yokoyama Shigeyuki; Nakashima Hideki; Yamamoto Naoki; Takaku Hiroshi(a)
AUTHOR ADDRESS: (a)Dep. Industrial Chem., Chiba Inst. Technol., Tsudanuma, Narashino, Chiba 275**Japan

JOURNAL: Biochemical and Biophysical Research Communications 211 (3):p 1041-1046 1995
ISSN: 0006-291X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We demonstrate the degradation of RNA bound to an antisense **oligonucleotide** by a reverse transcriptase enzyme-associated RNase H **activity**. We found that phosphorothioate **oligonucleotides** inhibit the RNase H **activity** by binding to AMV RT, rather than to the template RNA, whereas the RNase H **activity** of HIV-1 RT is not affected by the antisense phosphorothioate **oligonucleotide**. Selective inhibition of HIV-1 gene expression involves the degradation of the template RNA bound to the antisense phosphorothioate **oligonucleotide** by the RNase H **activity** associated with the HIV-1 polymerase.

8/3,AB/30 (Item 30 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

09821029 BIOSIS NO.: 199598275947
Site and mechanism of antisense inhibition by C-5 propyne **oligonucleotides**.
AUTHOR: Moulds Courtney; Lewis Jason G; Froehler Brian C; Grant Deborah; Huang Teresa; Milligan John F; Matteucci Mark D; Wagner Richard W(a)
AUTHOR ADDRESS: (a)Gilead Sci., 353 Lakeside Dr., Foster City, CA 94404** USA
JOURNAL: Biochemistry 34 (15):p5044-5053 1995
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Antisense gene inhibition occurs when an **oligonucleotide** (ON) has sufficient binding affinity such that it hybridizes its reverse complementary target RNA and prevents translation either by causing inactivation of the RNA (possibly by RNase H) or by interfering with a cellular process such as stalling a ribosome. The mechanisms underlying these processes were explored. Cellular antisense inhibition was evaluated in a microinjection assay using ON modifications which precluded or allowed in vitro RNase H cleavage of ON/RNA hybrids. RNase H-independent inhibition of protein synthesis could be achieved by targeting either the 5'-untranslated region or the 5'-splice junction of SV40 large T antigen using 2'-O-allyl phosphodiester ONs which contained C-5 propynylpyrimidines (C-5 propyne). Inhibition at both sites was 20-fold less **active** than inhibition using RNase H-competent C-5 propyne 2'-deoxy phosphorothioate ONs. In vitro analysis of association and dissociation of the two classes of ONs with complementary RNA showed that the C-5 propyne 2'-O-allyl phosphodiester ON bound to RNA as well as the C-5 propyne 2'-deoxy phosphorothioate ON. In vitro translation assays suggested that the two classes of ONs should yield equivalent antisense effects in the absence of RNase H. Next, ON/T antigen RNA hybrids were injected into the nuclei and cytoplasm of cells. Injection of C-5 propyne 2'-O-allyl phosphodiester ON/RNA hybrids resulted in expression, of T antigen, implying that the ONs dissociated from the RNA in cells which likely accounted for their low potency. In contrast, when C-5 propyne 2'-deoxy phosphorothioate ON/T antigen RNA complexes were injected into the nucleus, the duplexes were stable enough to completely block T-antigen translation, presumably by RNA inactivation. Thus, a dramatic finding is that C-5 propyne 2'-deoxy phosphorothioate ONs, once hybridized to RNA, are completely effective at preventing mRNA translation. The implication is that further increases in complex

stability coupled with effective RNase H cleavage will not result in enhanced potency. We predict that the development of more effective ONs will only come from modifications which increase the rate of ON/RNA complex formation within the nucleus.

8/3,AB/31 (Item 31 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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09661635 BIOSIS NO.: 199598116553
Oligonucleotides with novel, cationic backbone substituents: Aminoethylphosphonates.
AUTHOR: Fathi Reza(a); Huang Qing; Coppola George; Delaney William; Teasdale Rebecca; Krieg Arthur M; Cook Alan F
AUTHOR ADDRESS: (a)PharmaGenics Inc., 4 Pearl Court, Allendale, NJ 07401** USA
JOURNAL: Nucleic Acids Research 22 (24):p5416-5424 1994
ISSN: 0305-1048
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: **Oligonucleotide** (2-aminoethyl)phosphonates in which the backbone consisted of isomerically pure, alternating (2-aminoethyl)-phosphonate and phosphodiester linkages have been prepared and characterized. One of these single isomer **oligonucleotides** (Rp) formed a more stable duplex with DNA or RNA than its corresponding natural counterpart. Hybrid stability was more pH-dependent, but less salt-dependent than a natural duplex. The specificity of hybridization was examined by hybridization of an **oligonucleotide** containing one (2-aminoethyl)phosphonate to **oligonucleotides** possessing mismatches in the region opposite to the aminoethyl group. In contrast to **oligonucleotides** containing (aminomethyl)-phosphonate linkages, **oligonucleotide** (2-aminoethyl)phosphonates were completely stable to hydrolysis in aqueous solution. These **oligonucleotides** were resistant to nuclease **activity** but did not induce RNase H mediated cleavage of a complementary RNA strand. Incubation in a serum-containing medium resulted in minimal degradation over 24 hours. Studies of cell uptake by flow cytometry and confocal microscopy demonstrated temperature dependent uptake and intracellular localization. (2-aminoethyl)phosphonates represent a novel approach to the introduction of positive charges into the backbone of **oligonucleotides**.

8/3,AB/32 (Item 32 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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09618375 BIOSIS NO.: 199598073293
Photochemically and chemically **activatable** antisense **oligonucleotides**: Comparison of their reactivities towards DNA and RNA targets.
AUTHOR: Godard Gerard; Francois Jean-Christophe; Duroux Isabelle; Asseline Ulysse; Chassignol Marcel; Thuong Nguyen; Helene Claude(a); Saison-Behmoaras Tula
AUTHOR ADDRESS: (a)Lab. de Biophysique, INSERM U201-CNRS UA 481, 43 rue Cuvier 75005, Paris**France
JOURNAL: Nucleic Acids Research 22 (22):p4789-4795 1994
ISSN: 0305-1048
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Dodecadeoxyribonucleotides derivatized with 1,10-phenanthroline

or psoralen were targeted to the point mutation (G fwdarw U) in codon 12 of the Ha-ras mRNA. D[redacted]and RNA fragments, 27 nucleoti[redacted] in length, and containing the complementary sequence of the 12mers, we[redacted] used to compare the reactivity of the **activatable** dodecamers (cleavage of the target by the phenanthroline-12mer conjugates; photo-induced cross-linking of psoralen-12mer conjugates to the target). The reactivity of the RNA with the dodecamers was weaker than that of the DNA target. With psoralen-substituted **oligonucleotides**, it was possible to obtain complete discrimination between the mutated target (which contained a psoralen-reactive T(U) in the 12th codon) and the normal target (which contained G at the same position). When longer Ha-ras RNA fragments were used as targets (120 and 820 nucleotides), very little reactivity was observed. Part of the reactivity could be recovered by using 'helper' **oligonucleotides** that hybridized to adjacent sites on the substrate. A 'helper' chain length greater than 13 was required to improve the reactivity of dodecamers. However, the dodecanucleotides induced RNase H cleavage of the target RNA in the absence of 'helper' **oligonucleotide**. Therefore, in the absence of the RNase H enzyme, long **oligonucleotides** are needed to compete with the secondary structures of the mRNA. In contrast, formation of a ternary complex **oligonucleotide**-mRNA-RNase H led to RNAT cleavage with shorter **oligonucleotides**.

8/3,AB/33 (Item 33 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

09579560 BIOSIS NO.: 199598034478
Roles of RNase H in inhibition of SRS mouse leukemia virus (SRSV) expression by antisense **oligonucleotides**.
AUTHOR: Su Wenying; Shan Yifei
AUTHOR ADDRESS: Dep. Biophysics, Sch. Basic Med. Sci., Shanghai Med. Univ., Shanghai**China
JOURNAL: Acta Academiae Medicinae Shanghai 21 (5):p351-355 1994
ISSN: 0257-8131
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: Chinese; Non-English
SUMMARY LANGUAGE: Chinese; English

ABSTRACT: The **oligonucleotides** reported here were complementary to the primer region of 5' end (14 mer), env-LTR gene region (18 mer) and pol gene region (35 mer) of Mo-MuLV mRNA. They successfully inhibit the expression of SRSV mRNA in the rabbit reticulocyte lysate system. Among these, the 35 mer is the most efficiency inhibitor which % inhibition goes to 40% and the 14 mer is relatively less efficiency, which % inhibition is only 23%, RNase H could largely increase the effect of 35 mer which % inhibition were increased from 42% to 59%, it also increases the effect of 18 mer, where as it has made no effect on the 14 mer. Studies also proved this suppression of viral expression has sequence specific and dosage-dependent. Studies suggested that the possible mechanism of antisense **oligonucleotides** inhibit SRSV mRNA expression involved the attack of the **oligonucleotides**-RNA hybrid by RNase H **activity**. Additionally, studies here proved that a single strand DNA produced by asymmetric PCR could also inhibit the SRS mRNA expression.

8/3,AB/34 (Item 34 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

09408890 BIOSIS NO.: 199497417260
Selection of high-affinity RNA ligands to reverse transcriptase: Inhibition

of cDNA synthesis and RNase H **activity**.
AUTHOR: Chen Hang; Gold[redacted]y(a)
AUTHOR ADDRESS: (a)Dep. Mol., Univ. Colo., Boulder, CO 80[redacted]9**USA
JOURNAL: Biochemistry 33 (29):p8746-8756 1994
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Specific, high-affinity RNA ligands to avian myeloblastosis virus and Moloney murine leukemia virus reverse transcriptases were isolated from a combinatorial RNA library using the SELEX (systematic evolution of ligands by exponential enrichment) procedure. The selected RNA ligands bound their respective reverse transcriptases with approximately nanomolar dissociation constants. The ligands did not exhibit primary sequence conservation from selections against different target enzymes. Moreover, the selected ligands competed with the binding of template/primer complex and inhibited both the RNA-dependent DNA polymerase and the RNase H **activities** of the cognate reverse transcriptase. SELEX can yield both high-affinity and high-specificity **oligonucleotide** antagonists against specific members of a protein family.

8/3,AB/35 (Item 35 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

09390402 BIOSIS NO.: 199497398772
Antisense L/D-oligodeoxynucleotide chimeras: Nuclease stability, base-pairing properties, and **activity** at directing ribonuclease H.
AUTHOR: Damha Masad J(a); Giannaris Paul A; Marfey Peter
AUTHOR ADDRESS: (a)Dep. Chem., Otto Maass Chem. Build., McGill Univ., 801 Sherbrooke St. W., Montreal, Quebec, CAN **Canada
JOURNAL: Biochemistry 33 (25):p7877-7885 1994
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Ultraviolet thermal denaturation studies substantiate our earlier hypothesis that substitution of a L-nucleotide residue for a D-nucleotide within a DNA duplex permits a stable structure in which all bases are paired through Watson-Crick hydrogen bonds (Damha, M. J., Giannaris, P. A., Marfey, P., & Reid, L. S. (1991) Tetrahedron Lett. 32, 2573-2576). This conclusion is also evident from the NMR work of Blommers et al. (Blommers, M. J. J., et al. (1994) Biochemistry (following paper in this issue)). Our thermal denaturation studies indicate that, while weakening the interaction with target DNA and RNA, these substitutions allow for excellent cooperative binding. When the target is single-stranded DNA, the melting temperature of the complex is lowered by 4-5 degree C per L-dU incorporation and by 0.4-2.6 degree C when an internal D-dC is replaced by L-dC (1 M NaCl). When the target is RNA, the depression of T-m is also greater for L-dU substitutions (5-8 degree C) than for L-dC substitutions (2-4 degree C). The depressions of T-m, caused by introducing A/C and G/T mismatches at the same positions were significantly greater. L/D-DNA chimeras were found to **activate** RNase H cleavage when hybridized to RNA. Furthermore, the stability of chimeric L/D-DNA against degradation by various commercial phosphodiesterases was found to be significant, as was their stability against digestion in human serum. These experiments establish that L/D-DNA chimeras serve as excellent models of antisense **oligonucleotides**.

8/3,AB/36 (Item 36 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08902428 BIOSIS NO.: 199396053929
Growth inhibition of human tumor cell lines by antisense **oligonucleotides** designed to inhibit p120 expression.
AUTHOR: Perlaky Laszlo; Saijo Yasuo; Busch Rose K; Bennett C Frank; Mirabelli Christopher K; Crooke Stanley T; Busch Harris(a)
AUTHOR ADDRESS: (a)Dep. Pharmacol., Baylor Coll. Med., One Baylor Plaza, Houston, TX 77030**USA
JOURNAL: Anti-Cancer Drug Design 8 (1):p3-14 1993
ISSN: 0266-9536
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The human nucleolar antigen p120 was detected with an anti-p120 monoclonal antibody (MAbp120) in most human malignant tumors (Freeman et al., Cancer Research, 48, 1244-1251, 1988). Stable transfection of the sense p120 cDNA caused malignant transformation of NIH/3T3 cells in vitro, and the antisense p120 constructs markedly delayed the growth of these transformed cells (Perlaky et al., Cancer Research, 52, 428-436, 1992). Several p120 antisense phosphorothioate **oligonucleotides** designed to hybridize with different regions of the p120 sequence were screened on human tumor cell lines in vitro. Marked growth inhibition of HeLa, LOX and HRCC cell lines was found, particularly with antisense p120 **oligonucleotide** ISSI 3466 in combination with N-(1-(2,3-dioleyloxy)propy)-N,N,N-trimethylammonium chloride (DOTMA); **oligonucleotide** ISIS 3466 is complementary to a nontranslated region at the 3' end of the molecule. Preliminary in vivo studies on human LOX ascites tumor in nude mice showed marked inhibitory effects on tumor growth by the antisense **oligonucleotide** ISIS 3466 in the presence of DOTMA when treated on alternate days.

8/3,AB/37 (Item 37 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08899633 BIOSIS NO.: 199396051134
Phosphorothioate-phosphodiester **oligonucleotide** co-polymers: Assessment for antisense application.
AUTHOR: Ghosh Mridul K(a); Ghosh Krishnakali; Cohen Jack S
AUTHOR ADDRESS: (a)Cancer Pharmacol. Sect., Pharmacol. Dep., Georgetown Univ. Med. Center, Washington, DC 20007**USA
JOURNAL: Anti-Cancer Drug Design 8 (1):p15-32 1993
ISSN: 0266-9536
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Efforts have been made to reduce the disadvantages associated with the natural **oligonucleotides** (all-PO) for antisense application by introducing phosphorothioate (PS) linkages into the molecule. A series of such oligodeoxynucleotide copolymers (17-mers) complementary to the coding region of the rabbit beta-globin mRNA, and containing different proportions and arrangements of PO and PS bonds, were synthesized and tested for their protein-binding properties, nuclease stability in vitro, hybridizing ability with the complementary DNA (cDNA), ability to form RNase H-sensitive substrates and antisense **activity** in cell-free systems. The melting temperatures (T-m) of the co-polymers were reduced by up to 6 degree C relative to the all-PO oligo, compared to 11 degree C for the all-PS compound, indicating intermediate hybridizing abilities of the co-polymers. The

protein-binding studies with human serum albumin exhibited a linear correlation with the percentage of PS linkage present in the molecule. Nuclease susceptibilities of the co-polymers were also improved, but the number and position of the PS linkages played a significant role in such improvement. Translation inhibition by these **oligonucleotides** was only found in wheat germ agglutinin (WGA) extract, but not in rabbit reticulocyte lysate (PRL) cell-free system, suggesting the involvement of RNase H in their antisense **activities**. Provided they have gtoreq 50% PS linkages, the co-polymers produced almost the same increased inhibition in the WGA system as that of the all-PS oligo. The translation arrest in WGA extract is in good agreement with the in vitro cleavage found for rabbit globin mRNA in the oligo:mRNA duplex by RNase H alone. It is concluded that a copolymer of PO and PS might be preferable to either all-PO or all-PS for antisense applications.

8/3,AB/38 (Item 38 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08730849 BIOSIS NO.: 199395020200
Phosphorothioate **oligonucleotides** are inhibitors of human DNA polymerases and RNase H: Implications for antisense technology.
AUTHOR: Gao Wen-Yi; Han Fu-Sheng; Storm Christy; Egan William; Cheng Yung-Chi(a)
AUTHOR ADDRESS: (a)Dep. Pharmacol., Sch. Med., Yale Univ., 333 Cedar St., New Haven, Conn. 06510
JOURNAL: Molecular Pharmacology 41 (2):p223-229 1992
ISSN: 0026-895X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Phosphorothioate oligodeoxycytidine (S-dC-n) was used as a model compound to examine the impact of the number of phosphorothioate linkages and their position on the inhibition of human DNA polymerases and RNase H in vitro. S-dC-N with a chain length longer than 15 could inhibit human DNA polymerases and RNase H **activities**, in a linkage number-dependent manner. Longer oligomers were more potent inhibitors than shorter ones. Kinetic studies indicated that S-dC-28 was a competitive inhibitor of DNA polymerase alpha and beta with respect to the DNA template, whereas it was a noncompetitive inhibitor of polymerases gamma and delta S-dC-28 was also a competitive inhibitor of RNase H1 and H2 with respect to RNA-DNA duplex. Susceptibility of these enzymes to inhibition by S-dC-28 was in the order of delta apprxeq gamma gt alpha gt beta and RNase H1 gt RNase H2. Structural-**activity** relationships were explored with a group of S-dC-28 analogs that have phosphorothioate internucleotide linkages at various positions. The inhibitory effect depended on the total number of thioate linkages, rather than the position of the linkages within the oligomer or the chain length itself. No sequence specificity was found. In the presence of the complementary RNA, antisense phosphorothioates (S-oligos) exerted a biphasic effect on RNase H **activity**. At low concentrations S-oligos could enhance the cleavage of the RNA portion of S-oligo-RNA duplex, whereas at high concentrations (in excess of the complementary RNA) S-oligos could inhibit RNAse H and protect the complementary RNA from degradation. Together, these results suggest that the non-sequence-specific inhibitory effect of S-oligos should be taken into consideration in designing antisense inhibitors. This inhibitory **activity** could be avoided by decreasing the number of phosphorothioate linkages at the backbone, and S-oligos of 15-20 residues are preferable in antisense molecule design.

8/3,AB/39 (Item 39 from file: 5)

08726548 BIOSIS NO.: 199395015899
Requirements for strand transfer between internal regions of heteropolymer templates by human immunodeficiency virus reverse transcriptase.
AUTHOR: Destefano Jeffrey J; Mallaber Lisa M; Rodriguez-Rodriguez Lorna; Fay Philip J; Bambara Robert A(a)
AUTHOR ADDRESS: (a)Dep. Biochemistry, University Rochester, Rochester, N.Y. 14642
JOURNAL: Journal of Virology 66 (11):p6370-6378 1992
ISSN: 0022-538X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We have examined the ability of the reverse transcriptase (RT) from human immunodeficiency virus (HIV) to carry out strand transfer synthesis (i.e., switching of the primer to a new template) from internal regions of natural-sequence RNA. A 142-nucleotide RNA template (donor) primed with a specific 20-nucleotide DNA **oligonucleotide** was used to initiate synthesis. DNA **oligonucleotides** with homology to internal regions of the donor were used as acceptors. In this system, HIV RT produced strand transfer products. An HIV RT having RNase H depleted to 3% of normal (HIV RT-RD) catalyzed the transfer reaction inefficiently. An RNase H-minus deletion mutant of murine leukemia virus RT was unable to catalyze strand transfer. HIV RT-RD, however, efficiently catalyzed transfer when Escherichia coli RNase H was included in the reactions, while the mutant murine leukemia virus RT was not efficiently complemented by the E. coli enzyme. Evidently, RNase H **activity** enhances, or is required for, internal strand transfer. Two acceptors homologous to 27-nucleotide regions of the donor, one offset from the other by 6 nucleotides, were tested. The offset eliminated a sequence homologous to a prevalent DNA synthesis pause site in the donor. Strand transfer to this acceptor was about 25% less efficient, suggesting that RT pausing can enhance strand transfer. When the deoxynucleoside triphosphates in the reactions were reduced from 50 to 0.2 mu-M, increasing RT pausing, the efficiency of strand transfer also increased. A model for RT-catalyzed strand transfer consistent with

Mixed-backbone **oligonucleotides** as second generation antisense **oligonucleotides**: In vitro and in vivo studies.
AUTHOR: Agrawal Sudhir(a); Jiang Zhiwei; Zhao Qiuyan; Shaw Denise; Cai Qiuyin; Roskey Allysen; Channavajjala Lakshmi; Saxinger Carl; Zhang Ruiwen
AUTHOR ADDRESS: (a)Hybridon Inc., 620 Memorial Drive, Cambridge, MA 02139** USA
JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 94 (6):p2620-2625 1997
ISSN: 0027-8424
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Antisense **oligonucleotides** are being evaluated in clinical trials as novel therapeutic agents. To further improve the properties of antisense **oligonucleotides**, we have designed mixed-backbone **oligonucleotides** (MBOs) that contain phosphorothioate segments at the 3' and 5' ends and have a **modified** oligodeoxynucleotide or oligoribonucleotide segment located in the central portion of the **oligonucleotide**. Some of these MBOs indicate improved properties compared with phosphorothioate oligodeoxynucleotides with respect to affinity to RNA, RNase H **activation**, and anti-HIV **activity**. In addition, more acceptable pharmacological, in vivo degradation and pharmacokinetic profiles were obtained with these MBOs.

7/3,AB/4 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10828799 BIOSIS NO.: 199799449944
RNase H-independent antisense **activity** of **oligonucleotide** N3' fwdarw P5' phosphoramidates.
AUTHOR: Heidenreich Olaf; Gryaznov Sergie; Nerenberg Michael(a)
AUTHOR ADDRESS: (a)Nanogen, 10398 Pacific Center Court, San Diego, CA 92121 **USA
JOURNAL: Nucleic Acids Research 25 (4):p776-780 1997
ISSN: 0305-1048
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: **Oligonucleotide** N3' fwdarw P5' phosphoramidates are a new and promising class of antisense agents. Here we report biological properties of phosphoramidate **oligonucleotides** targeted against the human T cell leukemia virus type-I Tax protein, the major transcriptional transactivator of this human retrovirus. Isosequential phosphorothioate oligodeoxynucleotides and uniformly **modified** and chimeric phosphoramidate oligodeoxynucieotides containing six central phosphodiester linkages are all quite stable in cell nuclei. The uniformly **modified** anti-tax phosphoramidate oligodeoxynucleotide does not **activate** nuclear RNase H, as was shown by RNase protection assay. In contrast, the chimeric phosphoramidate-phosphodiester oligodeoxynucleotide is an efficient **activator** of RNase H. The presence of one or two mismatched nucleotides in the phosphodiester portion of **oligonucleotides** affected this **activation** only negligibly. When introduced into tax-transformed fibroblasts ex vivo, only the uniformly **modified** anti-tax phosphoramidate oligodeoxynucleotide caused a sequence-dependent reduction in the Tax protein level. Neither the chimeric phosphoramidate nor the phosphorothioate oligodeoxynucleotides significantly reduced tax expression under similar experimental conditions.

7/3,AB/5 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

```
? s rnase h

      S1      351  RNASE H
? s s1 and activ?

             351  S1
         3146867  ACTIV?
      S2     178  S1 AND ACTIV?
? s s2 and oligo?

>>>File 5 processing for OLIGO? stopped at OLIGORIBOADENYLATE
             178  S2
          230307  OLIGO?
      S3      63  S2 AND OLIGO?
? rd

...examined 50 records  (50)
...completed examining records
      S4      63  RD (unique items)
? s s2 and oligonucleotid?

             178  S2
           70402  OLIGONUCLEOTID?
      S5      46  S2 AND OLIGONUCLEOTID?
? rd

...completed examining records
      S6      46  RD (unique items)
? s s6 and (modify or modified)

              46  S6
           49683  MODIFY
          234613  MODIFIED
      S7       7  S6 AND (MODIFY OR MODIFIED)
? t s7/3,ab/all


 7/3,AB/1      (Item 1 from file: 5)
DIALOG(R)File   5:Biosis Previews(R)
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12053815   BIOSIS NO.: 199900334334
Extremely high and specific activity of DNA enzymes in cells with a
  Philadelphia chromosome.
AUTHOR: Warashina Masaki; Kuwabara Tomoko; Nakamatsu Yuka; Taira Kazunari
  (a)
AUTHOR ADDRESS: (a)National Institute for Advanced Interdisciplinary
  Research, AIST, MITI, Tsukuba Science City, 30**Japan
JOURNAL: Chemistry & Biology (London)  6 (4):p237-250 April, 1999
ISSN: 1074-5521
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Background: Chronic myelogenous leukemia (CML) results from
  chromosome 22 translocations (the Philadelphia chromosome) that creates
  BCR-ABL fusion genes, which encode two abnormal mRNAs (b3a2 and b2a2).
  Various attempts to design antisense oligonucleotides that
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specifically cleave abnormal L6 BCR-ABL fusion mRNA have not been successful. Because b[redacted] mRNA cannot be effectively cl[redacted]ed by hammerhead ribozymes near the BCR-ABL junction, it has proved very difficult to engineer specific cleavage of this chimeric mRNA. Nonspecific effects associated with using antisense molecules make the use of such antisense molecules questionable. Results: The usefulness of DNA enzymes in specifically suppressing expression of L6 BCR-ABL mRNA in mammalian cells is demonstrated. Although the efficacy of DNA enzymes with natural linkages decreased 12 hours after transfection, partially **modified** DNA enzymes, with either phosphorothioate or 2'-O-methyl groups at both their 5' and 3' ends, remained **activefor** much longer times in mammalian cells. Moreover, the DNA enzyme with only 2'-O-methyl modifications was also highly specific for abnormal mRNA. Conclusions: DNA enzymes with 2'-O-methyl modifications are potentially useful as gene-inactivating agents in the treatment of diseases such as CML. In contrast to conventional antisense DNAs, some of the DNA enzymes used in this study were highly specific and cleaved only abnormal BCR-ABL mRNA.

7/3,AB/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11832887 BIOSIS NO.: 199900078996
Cellular penetration and antisense **activity** by a phenoxazine-substituted heptanucleotide.
AUTHOR: Flanagan W Michael(a); Wagner Richard W; Grant Deborah; Lin Kuei-Ying; Matteucci Mark D(a)
AUTHOR ADDRESS: (a)Gilead Sci. Inc., Foster City, CA 94404**USA
JOURNAL: Nature Biotechnology 17 (1):p48-52 Jan., 1999
ISSN: 1087-0156
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: One of the major barriers to the development of antisense therapeutics has been their poor bioavailability. Numerous **oligonucleotide** modifications have been synthesized and evaluated for enhanced cellular permeation with limited success. Phenoxazine, a tricyclic 2'deoxycytidine analog, was designed to improve stacking interactions between heterocycles of **oligonucleotide**/RNA hybrids and to enhance cellular uptake. However, the bioactivity and cellular permeation properties of phenoxazine-**modified** **oligonucleotides** were unknown. Incorporation of four phenoxazine bases into a previously optimized C5 propyne pyrimidine **modified** 7-mer phosphorothioate **oligonucleotide** targeting SV40 large T antigen enhanced in vitro binding affinity for its RNA target and redirected RNAse H-mediated cleavage as compared with the 7-mer C-5 propynyl phosphorothioate **oligonucleotide** (S-ON). The phenoxazine/C-5 propynyl U 7-mer S-ON showed dose-dependent, sequence-specific, and target-selective antisense **activity** following microinjection into cells. Incubation of the phenoxazine/C-5 propynyl U S-ON with a variety of tissue culture cells, in the absence of any cationic lipid, revealed unaided cellular penetration, nuclear accumulation, and subsequent antisense **activity**. The unique permeation properties and gene-specific antisense **activity** of the 7-mer phenoxazine/C-5 propynyl U S-ON paves the way for developing potent, cost-effective, self-permeable antisense therapeutics.

7/3,AB/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10856087 BIOSIS NO.: 199799477232